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Supercritical Fluid Extraction and Fractionation of Corn Bran Oil

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Supercritical fluid extraction (SFE) has been combined with supercritical fluid chromatography (SFC) in an analytical mode to develop a system for fractionating and enriching high value ferulate-phytosterol esters (FPE) contained in corn bran oil. Corn bran was initially extracted with neat supercritical carbon dioxide (SC-CO_2) with all possible combinations of pressures (13.8, 34.5, 69 MPa) and temperatures (40, 60, 80° C) to see if the FPE could be enriched in the extracts. These initial studies showed the greatest percentage of FPE could be extracted under two sets of conditions: 69 MPa/80° C and 34.5 MPa/40° C. Both sets of parameters yielded an extract containing ~1.25 % FPE. A stock supply of corn bran oil was then produced by scaled-up SFE at 34.5 MPa and 40° C for subsequent chromatographic fractionation.

The SFE-obtained corn bran oil was then applied to the head of a chromatographic column containing an amino-propyl sorbent. SFC was commenced with neat SC-CO_2 at 69 MPa and 80° C to remove the majority of the triglyceride-based oil. The pressure and temperature were then lowered to 34.5 MPa and 40° C, respectively, and ethanol was added as a modifier. The modifier was added in an increasing stepwise gradient program and fractions were collected at equal volume intervals. The resultant fractions were analyzed by high-performance liquid chromatography with evaporative light-scattering detection and showed that FPE could be enriched to a 14.5 wt % level.

Introduction

It has been reported that the hexane extract derived from corn bran contains high levels of ferulate-phytosterol esters (FPE), similar in composition and function to oryzanol which is found in rice bran and rice bran oil.[1-3] Oryzanol has been shown to lower the levels of serum cholesterol in laboratory animals and man.[4,5] Recently, ferulate-phytosterol esters, and in particular the sitostanyl ester, have also been shown to have cholesterol-lowering activity.[6-7]

Corn bran and corn fiber are obtained as by-products from the dry- and wet-milling of corn, respectively, processes that are used in converting corn into numerous products. Moreau et al.[8] reported that both corn bran and corn fiber yield oils that contain FPE. They performed hexane and SC-CO_2 extractions and found that the corn fiber oil contained higher percentages (3-6 wt %) of FPE than corn bran oil (1.5 wt %). They also noted that corn fiber produced lower levels of oil than corn bran.

In this study, we have expanded upon earlier research that has incorporated a two-step process consisting of supercritical fluid extraction and supercritical fluid chromatography combined, to fractionate and enrich high value nutraceuticals.[9-11] We have developed an integrated procedure for the extraction of corn bran and combined it with supercritical fluid chromatography to obtain fractions enriched in the ferulate-phytosterol esters. These trials utilized analytical-scale equipment for designing and optimizing the fractionation process, but the knowledge gained in these studies will be applied to future scaled-up runs. With respect, processing parameters such as pressure, temperature, modifier and sorbent type were examined.

Experimental Section

Corn Bran

The corn bran was a generous gift from Mr. Rex Winter of Illinois Cereal Mills, Inc. (Paris, IL).

Supercritical fluid extraction

The SFE screening studies were performed with an Isco, Inc. Model SFX 3560 automated extractor (Lincoln, NE). Corn bran was extracted with all possible combinations of three pressures (13.8, 34.5 and 69 MPa) and three temperatures (40, 60 and 80° C). The extractions

were conducted for 120 minutes at a pump delivery flow rate of 2 mL/min, liquid CO_2. The restrictor was heated to 80°C, with utilization of the pressurized collection at 0° C.

Corn bran oil stock supplies were obtained by extraction with SC-CO_2 at 34.5 MPa/40° C and 69 MPa/80° C using the National Center for Agricultural Utilization Research SFE pilot plant.[12] The extract derived from the lower pressure/temperature SFE was centrifuged to separate the oil from the waxes in the extract. The separated oil was decanted into a funnel and filtered through glass wool. The resultant oil was then stored at 4° C until use. SFE at 69 MPa/80° C yielded an extract that contained oil, waxes and a considerable amount of water. It also had to be centrifuged to separate the layers. The oil was pipetted off, filtered through glass wool and stored at 4° C.

Supercritical fluid fractionation

Supercritical fluid fractionation (SFF) studies were also performed with an Isco, Inc. Model SFX 3560 automated extractor (Lincoln, NE). The sorbents tested for the SFF were as follows: silica gel (60-200 mesh, J.T. Baker Chemical Co., Phillipsburg, NJ), amino-propyl bonded silica (40 μm, Varian Associates Inc., Harbor City, CA), neutral alumina (60-325 mesh, Fisher Scientific, Fair Lawn, NJ) and diol (37-55 μm, Millipore Corporation Waters Chromatography, Milford MA). They were added to a 10 mL extraction vessel and corn bran oil (~0.4 g) was manually applied to the top of the sorbent bed. The extraction/fractionation procedure was then commenced with fractions collected at timed intervals (see below). The first fraction was intended to recover most of the triglyceride-based oil. The parameters for subsequent fractions were designed to fractionate and enrich the collection of FPE.

Parameters applied for a typical SFF run are as follows:

Fraction 1	69.0 MPa	80°C	60 min	2 mL/min	CO_2
Fraction 2	34.5 MPa	40°C	60 min	2 mL/min	1 % EtOH/CO_2
Fraction 3	34.5 MPa	40°C	60 min	2 mL/min	2 % EtOH/CO_2
Fraction 4	34.5 MPa	40°C	60 min	2 mL/min	3 % EtOH/CO_2
Fraction 5	34.5 MPa	40°C	60 min	2 mL/min	5 % EtOH/CO_2
Fraction 6	34.5 MPa	40°C	60 min	2 mL/min	7 % EtOH/CO_2
Fraction 7	34.5 MPa	40°C	60 min	2 mL/min	10 % EtOH/CO_2
Fraction 8	34.5 MPa	40°C	60 min	2 mL/min	15 % EtOH/CO_2
Fraction 9	34.5 MPa	40°C	30 min	2 mL/min	20 % EtOH/CO_2

High-performance liquid chromatography

All analyses were 15 µL injections of 5 mg/mL solutions. They were performed using a Spectra-Physics SP8800 pump connected to a SpectraSYSTEM AS3000 autosampler equipped with a Rheodyne 7010-151 loop injector (100 µL) (Thermo Separation Products, Inc., San Jose, CA), a Bio-Rad Model 1250424 column heater (Bio-Rad, Inc., Richmond CA) @ 30°C, an Alltech Model 500 ELSD evaporative light-scattering detector (ELSD) at 40°C and 1.5 SLPM of N_2 (Alltech Associates, Inc., Deerfield, IL), and a ChromQuest Chromatography Data System (ThermoQuest, Inc., San Jose, CA). The HPLC column was a Chromsep Cartridge, Lichrosorb DIOL, 5µm, 3 x 100 mm (Chrompack, Raritan, NJ). The mobile phase was a linear gradient of solvent A (hexane:acetic acid, 1000:1, v:v) and solvent B (hexane:2-propanol, 100:1, v:v) at a flow rate of 0.5 mL/min. The linear gradient timetable was: at 0 min, 100/0; at 8 min 100/0; at 10 min, 75/25; at 40 min, 75/25; at 41 min, 100/0; at 50 min, 100/0 (%A/%B, respectively).

Results and Discussion

The initial SC-CO_2 extractions were performed to check if ferulate-phytosterol esters were preferentially extracted from the corn bran. As can be seen in Table 1, the FPE ranged between 0.67 - 1.26 wt % of the extracts, indicating that they were not selectively extracted. All major classes of lipid-type compounds were present in each extract.

Table 1. Weight % and Mass Recoveries of Components From the SFE of Corn Bran

Compound	**13.8 MPa/80° C**		**13.8 MPa/60° C**		**13.8 MPa/40° C**	
	Weight %	**Mass (mg)**	**Weight %**	**Mass (mg)**	**Weight %**	**Mass (mg)**
FASE[a]	19.85	0.10	6.89	0.40	3.92	3.41
TG[b]	53.40	0.27	60.95	3.54	90.11	78.49
FFA[c]	23.68	0.12	28.23	1.64	4.04	3.52
FS[d]	2.40	0.01	3.19	0.19	1.10	0.96
FPE[e]	0.67	0.01	0.74	0.04	0.83	0.72
	34.5 MPa/80° C		**34.5 MPa/60° C**		**34.5 MPa/40° C**	
	Weight %	**Mass (mg)**	**Weight %**	**Mass (mg)**	**Weight %**	**Mass (mg)**
FASE	3.10	2.79	3.60	3.75	3.15	3.46

TG	87.40	78.40	87.67	91.44	90.91	100.09
FFA	3.83	3.44	4.88	5.09	3.57	3.93
FS	4.62	4.14	2.73	2.85	1.15	1.26
FPE	1.04	0.94	1.12	1.17	1.23	1.35

	69 MPa/80° C		**69 MPa/60° C**		**69 MPa/40° C**	
	Weight %	**Mass (mg)**	**Weight %**	**Mass (mg)**	**Weight %**	**Mass (mg)**
FASE	4.10	4.32	3.48	3.24	3.67	2.99
TG	88.96	93.86	88.16	81.99	87.26	71.11
FFA	4.54	4.80	5.40	5.02	3.55	2.89
FS	1.13	1.20	2.05	1.91	4.44	3.62
FPE	1.26	1.33	0.92	0.85	1.09	0.89

a) FASE = fatty acid-phytosterol esters, b) TG = triglycerides, c) FFA = free fatty acids, d) FS = free sterols, e) FPE = ferulate-phytosterol esters

Table 1 also shows the extraction parameters which yield the most enrichment of FPE. This is noted in weight percent of the extract and by mass. There were two sets of extraction parameters that produced almost identical results: 34.5 MPa/40° C and 69 MPa/80° C. SFE at 34.5 MPa/40° C yielded an extract containing 1.23 wt % FPE which was equivalent to 1.35 mg of a total extract of 110.1 mg. SFE at 69 MPa/80° C produced an extract containing 1.26 wt % FPE which equated to 1.33 mg from a total extract of 105.5 mg. These two sets of pressure/temperature combinations were then used for the production of two stock supplies of corn bran oil employing a SFE pilot plant. Corn bran oil produced by SFE at 34.5 MPa/40° C was then utilized for the SFF studies.

For the SFF experiments, an extraction cell was filled with sorbent and the corn bran oil was applied to the top (inlet) of the cell. SFF was commenced with neat CO_2, and then ethanol (EtOH) was added in a stepwise gradient to effect elution of the corn bran oil components. Ethanol was selected as the cosolvent because it enjoys GRAS (Generally Regarded As Safe) status in the United States for its use in food processing.

SFF trials using silica gel yielded mass balances where ~95 % of the starting corn bran oil was recovered. The vast majority (~90 %) of the triglyceride portion of the oil eluted in the first three fractions. However, the overall FPE recovery was low (42 %) and partitioned over many fractions, rather than being concentrated in one particular fraction.

Supercritical fluid fractionation with diol sorbent provided nearly a total mass balance recovery (98 %) with ~ 94 % of the triglycerides eluting in the first fraction. However, about half (46 %) of the FPE also eluted in the first fraction. The FPE then proceeded to elute in the subsequent fractions. The total FPE recovery was just over 80 %.

SFF data obtained using neutral alumina as the fractionating sorbent proved to be complex. Mass recovery was only about 2/3 of the starting material, and individual component recovery was erratic: triglyceride recovery was low (17.5 %), but diglyceride and free fatty acid recoveries were substantially elevated. This may not be all that unexpected as earlier research by King et al.[13] reported chemical reactions occurring with an alumina sorbent. They noted that transesterification of triglycerides occurred using SC-CO_2 over a packed bed of alumina that had been pretreated with methanol. Also, FPE recovery was about 5X the starting amount, a result difficult to account for.

The amino-propyl sorbent was found to afford the best fractionation/enrichment of corn bran oil and the fractionation was able to be simplified to four steps: neat CO_2, 1 vol % EtOH/CO_2, 2 vol % EtOH/CO_2, 10 vol % EtOH/CO_2. Total mass balance of the components in the extract was demonstrated and 90 % of the triglycerides eluted in the first fraction. All of the FPE were recovered with 99 % contained in a single fraction (10 vol % EtOH/CO_2).

Since these fractionation/enrichment studies would be used for future research on a semi- or preparative-scale, it was decided to conduct multiple supercritical fluid fractionation experiments using the same sorbent bed. The required maximum sample load was not examined, but these experiments were required since it would be necessary to use the sorbent at least several times to prevent the generation of large amounts of waste sorbent.

The same amino-propyl sorbent bed was tested for five SFF runs employing ~0.4 g of corn bran oil each time. A sorbent reconditioning step was incorporated between each fractionation. It consisted of SC-CO_2 at 69 MPa and 80° C at a flowrate of 2 mL/min for 60

minutes to remove any excess EtOH from the sorbent charge. As shown in Table 2, the data are very encouraging.

Table 2. Percent Recoveries from Corn Bran Oil Using Supercritical Fluid Fractionation with Amino-propyl Sorbent

Mass	FASE[a]	TG[b]	FFA[c]	FS[d]	1,3 DG[e]	FPE[f]	1,2 DG[g]
Individual Runs							
101.87	114.94	102.19	77.11	105.44	123.65	101.60	107.96
101.02	95.58	102.45	85.84	102.00	111.91	96.11	78.15
98.85	95.05	98.96	85.84	127.01	133.68	106.53	93.27
98.91	95.90	99.72	77.33	116.85	115.76	106.73	107.00
95.61	93.77	95.70	78.95	121.94	133.92	106.66	94.71
Average Recoveries							
99.25	99.05	99.80	81.01	114.65	123.78	103.53	96.22

a) FASE = fatty acid-phytosterol esters, b) TG = triglycerides, c) FFA = free fatty acids, d) FS = free sterols, e) 1,3 DG = 1,3 diglycerides, f) FPE = ferulate-phytosterol esters, g) 1,2 DG = 1,2 diglycerides

The average mass balance showed approximately total recovery (99.25 %) and the FPE yielded an average recovery of 103.5 %. The occurrence of ferulate-phytosterol ester recovery over 100 % is not surprising when the small FPE mass isolated is taken into consideration, and considering the possibility of weighing errors and chromatographic integration errors.

The first fraction contained the majority of the fatty acid-phytosterol esters and triglycerides and for the five SFF runs averaged 89.2 wt % of the mass. The fourth fraction mainly consisted of free fatty acids, free sterols, diglycerides and ferulate-phytosterol esters and for the five SFF runs averaged 9.2 wt % of the mass. The FPE were collected in the third (2 vol % EtOH/CO_2) and fourth (10 vol % EtOH/CO_2) fractions. However, they were concentrated in the latter, where on average, 99.6 wt % were present. The FPE composed on the average 14.5 wt % of the fourth fraction for the five SFF runs, whereas they only represented 1.29 wt % of the

starting corn bran oil. Thus, an enrichment factor of 11.24 can be attained from this fractionation/enrichment process.

The individual runs depict a small but noticeable decline in mass recovery for the fifth SFF run, and this may indicate a slight decline in sorbent efficiency. However, the individual components of the corn bran oil show good recoveries except for the free fatty acids, free sterols and 1,3 diglycerides. In retrospect, the decrease in mass recovery may be due to an instrumentation error in collection efficiency. Instrumentation error is proposed because worn extraction chamber seals were replaced after the fifth run and a small but noticeable amount of oil was found when the extraction chamber was cleaned, thus indicating a small loss of sample.

The high recoveries associated with the free sterols and the 1,3 diglycerides warrant further investigation. This could possibly be related to the chromatography system which is not specifically optimized for their analysis. The peaks tended to be broad and had shoulder peaks, and thus not ideal peak shape for chromatographic analysis. Likewise, the low recoveries of free fatty acids are still unaccounted for.

It was noted earlier that a sorbent reconditioning step was carried out between each supercritical fluid fractionation experiment. Fractions collected during these reconditioning runs yielded an average of 0.46 mg. Therefore, carryover from one run to the next does not seem to be problematic.

Conclusions

In this study, we have successfully demonstrated a two-step process of supercritical fluid extraction and supercritical fluid chromatography on an analytical-scale to enrich and fractionate ferulate-phytosterol esters from corn bran oil. These studies, employing analytical-scale equipment have provided valuable information which will be needed for experimental scale-up. In addition, only environmentally-benign (carbon dioxide) and GRAS solvents (ethanol) have been used for the extraction and fractionation (chromatography) of these potential nutraceuticals and high value components.

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